A Novel Look into Estrogen Receptor–Negative Breast Cancer Prevention with the Natural, Multifunctional Signal Transduction Inhibitor Deguelin

Perspective on Murillo et al., p. 942

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Abstract This perspective on Murillo et al. (beginning on page 942 in this issue of the journal) examines the potential of the naturally derived agent deguelin to prevent mammary tumorigenesis. These investigators showed that deguelin inhibits wnt/β-catenin signaling in breast cancer cell lines, in addition to inhibiting other previously reported signaling pathways. Our growing understanding of deguelin mechanisms could lead to important advances in the prevention of estrogen receptor–negative breast and other cancers.

It is estimated that approximately 192,370 new cases of and 40,170 deaths from breast cancer will occur in 2009 (1). Recent advances in targeted drug development have successfully prevented or treated estrogen receptor (ER)–positive breast cancers by interfering with estrogen signaling or production. The selective ER modulator tamoxifen has been shown to prevent ER-positive breast cancer in high-risk women (2), reducing overall breast cancer incidence by 50% in the Breast Cancer Prevention Trial (2). In the Study of Tamoxifen and Raloxifene (STAR), the selective ER modulator raloxifene equaled tamoxifen in preventing invasive breast cancer, but with fewer side effects (3). Neither drug, however, prevented ER-negative breast cancer, which generally is more aggressive than ER-positive disease and accounts for 30% of all breast cancers. Therefore, it is imperative to discover agents that can improve our ability to prevent and/or treat breast cancer, particularly ER-negative breast cancer.

Naturally derived compounds are of particular interest for cancer prevention because they are readily available and may be less toxic than synthetic drugs. Green tea has anticarcinogenic activity in multiple organ systems, and the catechins in green tea reduced the formation of tumor blood vessels in xenograft models of ER-negative breast cancer (4). Samy et al. (5) showed that the natural agent luteolin, a flavonoid found in green vegetables, inhibited 7,12-dimethylbenz(*a*)anthracene–induced mammary tumors in rats. The isoflavones have been studied intensively for potential cancer-preventive effects, and soybeans and other nonfermented soy products are the most important source of dietary isoflavones. Epidemiologic studies have shown that breast cancer incidence is inversely related to soy intake in Asians of the Far East and Asian Americans (6). There is a concern, however, that the soy isoflavones, which are phytoestrogens, may stimulate existing breast cancer because of their estrogen agonist (albeit weak) activities (7). Countering this concern, four recent trials showed that daily intake of 36 to 100 mg of soy did not cause any increase in breast epithelial cell proliferation among breast cancer patients or healthy individuals (8–11).

Berries are another promising source of cancer-preventive agents. Freeze-dried black raspberries and strawberries are capable of inhibiting colon, esophagus, and oral cavity tumor formation in animal models of chemically induced carcinogenesis (12–14). Olsson et al. (15) showed that berry extracts can inhibit the proliferation of both colon and breast cancer cells, and Seeram et al. (16) showed that an extract from multiple berries can inhibit the growth and induce the apoptosis of breast MCF-7 and other cancer cell lines.

In this issue of the journal, Murillo et al. (17) report their study of the promising natural agent deguelin in ER-negative breast cancer cells. Deguelin is a rotenoid and is isolated from African *Mundulea sericea* (Leguminosae; ref. 18). Several *in vitro* and *in vivo* studies have shown that deguelin is a promising cancer-preventive and therapeutic agent. The first reported potential chemopreventive activities of deguelin came from the work of Pezzuto et al. in mammary gland and skin carcinogenesis models (18, 19). Several subsequent studies indicated the potential of deguelin for lung cancer prevention. Chun et al. (20) showed that deguelin sensitized both premalignant and malignant human bronchial epithelial cells to apoptosis through phosphatidylinositol 3-kinase (PI3K)/AKT inhibition and did not affect normal bronchial epithelial cells. Lee et al. (21) of the same group later showed that deguelin suppressed AKT signaling that normally is enhanced by tobacco carcinogens in a mouse model of lung carcinogenesis. Therapeutically, deguelin has been shown to regulate various signaling pathways and thus can affect growth, apoptosis,

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and/or angiogenesis in several preclinical cancer systems, including those of colon, prostate, lung, head and neck, stomach, and/or breast cancer (22–30). Deguelin has inhibited mammalian target of rapamycin–mediated expression of survivin, an antiapoptotic factor, by upregulating AMP-activated kinase signaling and inhibiting AKT signaling in both premalignant and malignant lung cells (22, 23). Peng et al. (29) also showed that deguelin inhibited survivin expression, which enhanced the chemosensitivity of breast cancer cells. Deguelin inhibition of AKT also has been reported to attenuate radiation-induced AKT signaling, which enhanced sensitivity to the cytotoxic effects of radiotherapy, in MDA-MB-231 ER-negative breast cancer cells (30)

Deguelin also has been implicated as an inhibitor of other molecular pathways critical for tumorigenesis. Nair et al. (26) found that deguelin inhibits the activation of the nuclear factor κB (NF-κB) pathway. These investigators determined that deguelin specifically inhibited the phosphorylation and activation of inhibitor of κBα, leading to the degradation of inhibitor of κBα and suppression of NF-κB–regulated expression of genes key to cellular survival. Cyclooxygenase-2 is another signal transduction molecule affected by deguelin. Lee et al. (25) showed that deguelin inhibited PI3K/AKT–induced cyclooxygenase-2 expression in premalignant human bronchial epithelial and non–small-cell lung cancer cells. This group also showed that deguelin treatment increased the expression levels of the proapoptotic proteins p53, p21, and p27. Oh et al. (27, 28) showed novel antiangiogenic activities of deguelin in targeting hypoxia-inducible factor 1 (HIF-1). This group showed that deguelin suppressed PI3K/AKT–induced *de novo* synthesis of HIF-1α protein and reduced the half-life of the synthesized HIF-1α protein through inhibition of heat shock protein-90 chaperon function. Deguelin inhibition of heat shock protein-90 function and HIF-1α protein expression also has been reported to attenuate the radioresistance of lung cancer cells (24).

Murillo et al. (17) now report that deguelin inhibits another critical pathway in breast cells, the wnt/β-catenin pathway. These investigators showed that deguelin inhibited the growth of breast cancer cell lines that have both ER-positive and ER-negative characteristics and did so most strongly in ER-negative MDA-MB-231 cells. The effects on ER-negative disease are particularly interesting because only a few agents have been shown to inhibit the growth of ER-negative breast cancer cell lines. Previous studies have shown that retinoid X receptor–selective retinoids, such as bexarotene, and epidermal growth factor receptor or Her2 tyrosine kinase inhibitors, such as gefitinib and lapatinib, prevent the formation of ER-negative breast cancers in MMTV-ErbB2 transgenic mouse models (31, 32). Murillo et al. now show that deguelin causes a cell cycle blockade and induces apoptosis.

Their investigation of deguelin effects in MDA-MB-231 cells involved the use of microarray analysis to identify novel deguelin targets that could be examined in future cancer prevention and/or therapy studies. This microarray analysis confirmed that deguelin regulates many critical pathways, including the G_1-S phase cell cycle check point and apoptosis, NF-κB, and the p38 mitogen-activated kinase/c-Jun NH_2-terminal kinase pathways, in breast cancer cells. This analysis also unexpectedly found that deguelin modulated the wnt/β-catenin pathway in breast cancer cells. The major new findings on deguelin regulation of the wnt pathway are illustrated in Fig. 1, along with previously known mechanisms of deguelin regulation of signal transduction. Deguelin downregulated several of the important activators (WNT2B, WNT3, and WNT14) of wnt signaling and upregulated two major inhibitors (WIF1 and DKK4) of the wnt/β-catenin pathway. In addition, deguelin caused a decrease in β-catenin expression, and Murillo et al. suggest that deguelin targeted β-catenin for degradation by decreasing the inactive form of glycogen synthase kinase 3β. The observed reduction in β-catenin expression could be particularly significant because β-catenin expression is increased in aggressive breast cancer cell lines versus in immortalized normal MCF10F cells (33). If deguelin could prevent β-catenin from increasing to dangerous levels in women at risk of breast cancer, then this natural product or other agents targeting similar pathways may be useful for preventing the most aggressive forms of breast cancer.

Overall, Murillo et al. confirmed that deguelin affects many signal transduction pathways and discovered the mechanistic importance of the wnt/β-catenin pathway to deguelin effects. Characterizing the many targets inhibited by deguelin identifies signaling pathways critical for tumorigenesis as well as potential resistance pathways. This work highlights the opportunity to study combinations of deguelin with other inhibitors of the AKT, NF-κB, and now the wnt pathways to enhance the potential of deguelin for preventing ER-negative and other breast cancers.

To extend the observations of Murillo et al., it will be important to examine the effects of deguelin on normal, premalignant, and breast cancer cell lines, particularly ER-negative cell lines, other than those included in these investigators' report: the ER-positive breast cancer cell lines MCF-7, T47D, and BT474; the ER-negative breast cancer cell line MDA-MB-231; and the immortalized, normal mammary epithelial cell line MCF12F. Studying the effects of deguelin on an expanded set of breast cell lines could determine which molecular subtype of breast cancer would respond best to deguelin treatment. It would be particularly important to determine whether other ER-negative cell lines are as sensitive to deguelin as were the triple (ER, HER2, and progesterone receptor)-negative MDA-MB-231 cells. Although Murillo et al. included immortalized normal mammary epithelial MCF12F cells in their analyses, they focused mainly on breast cancer cell lines. Therefore, it would be important to use nonimmortalized, normal mammary epithelial cells such as human mammary epithelial cells in future studies to gain a better understanding of the effects of deguelin on normal breast cells.

Most important, future studies will determine the *in vivo* effects of deguelin. For example, because these investigators' main finding was that deguelin modulated the wnt/β-catenin signaling pathway, it is essential to determine whether deguelin would prevent breast cancer in *wnt-1* transgenic mice, which develop ER-negative breast cancer within the first year of life (34). In addition to *wnt-1* transgenic mice, the *MMTV-ErbB2* and *p53*-null mouse models also have been used to investigate the effects of novel chemopreventive agents on ER-negative breast cancer development. Studies in these animal models will be necessary to advance our understanding of deguelin for preventing ER-negative and other breast cancers. It is also imperative to assess the potential toxicity of deguelin in these animal models. A potential barrier to the use of

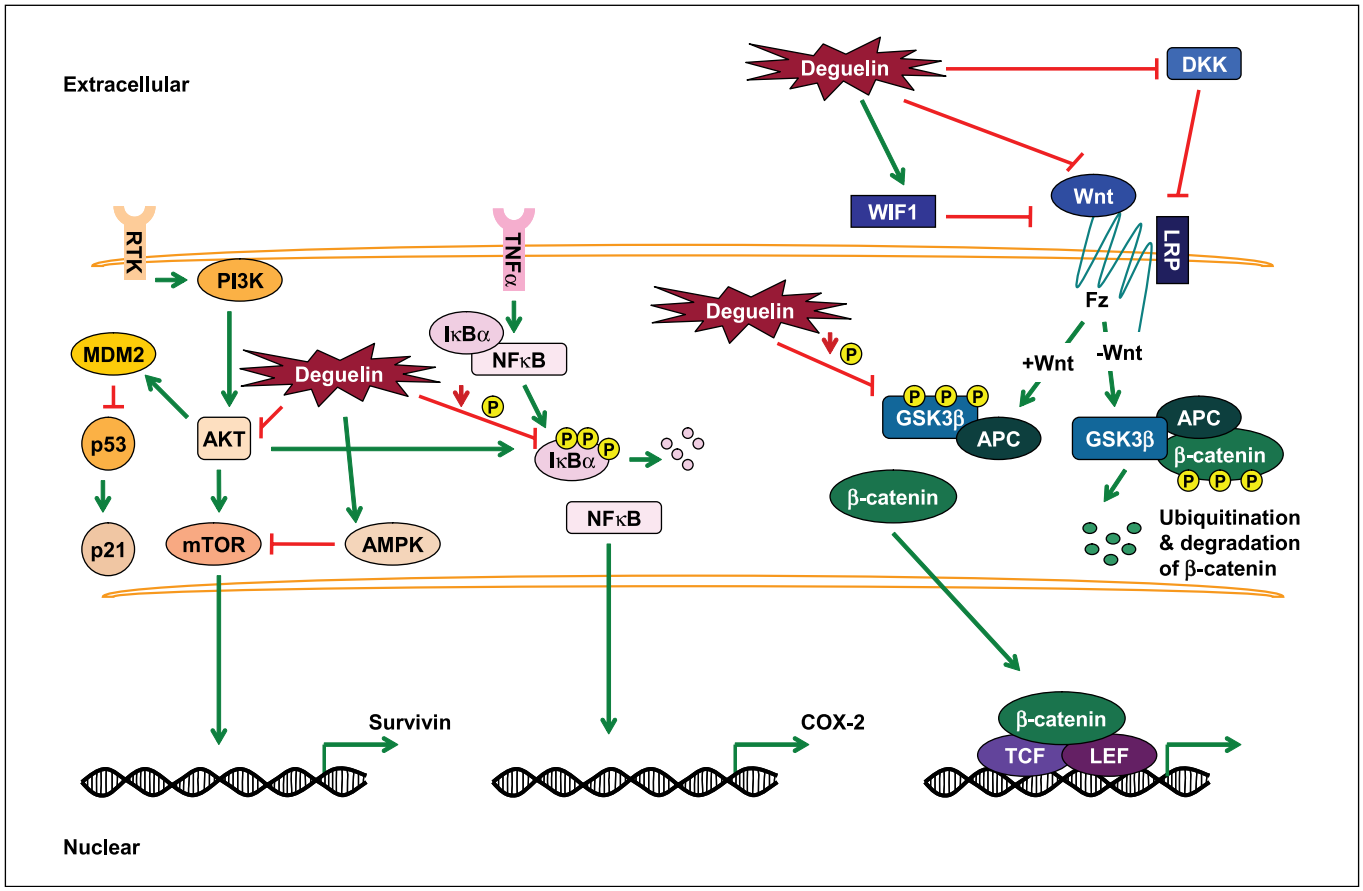

Fig. 1. Proposed mechanisms of action of deguelin. It has been shown previously that deguelin inhibits the PI3K/AKT and NF-κB signaling pathways in regulating gene expression (*left side*; refs. 18, 20–22, 25–29, 37–39). As shown by the novel findings of Murillo et al. reported in this issue of the journal, deguelin also regulates the wnt/β-catenin pathway (*right side*).

deguelin is the possible risk of a Parkinson's disease–like syndrome, which has been reported in rats treated with a high dose of deguelin (35). Woo et al. (36) recently suggested using deguelin in a liposomal formulation to improve therapeutic efficacy and reduce potential side effects. Kim et al. (37) of the same group also reported novel derivatives of deguelin with several potentially superior features (versus the parent compound) for clinical use. These novel deguelin-based treatments could provide strong preventive and therapeutic effects with minimized potential side effects.

Nonetheless, the present study of Murillo et al. makes a significant contribution by further characterizing the molecular pathways that are regulated by deguelin. In the future, it will be important to determine which types of breast cancer can be treated or prevented by deguelin and whether deguelin should be used in combination with other cancer-preventive or therapeutic agents. Furthermore, it is imperative to continue to study the many molecular pathways affected by deguelin to identify which ones should be targeted for optimal therapeutic and preventive effects and which ones may contribute to resistance and toxicity. The collective studies of deguelin have shown that this promising natural product regulates multiple molecular pathways and may be useful for preventing and treating ER-negative breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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